

A Prospective Study of Estradiol and Breast Cancer in Japanese Women¹

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Abstract

Few studies have prospectively examined endogenous hormone levels as risk factors for breast cancer. The present study compares prediagnostic hormone levels using stored serum from breast cancer cases and controls selected from the Life Span Study population of the Radiation Effects Research Foundation in Hiroshima and Nagasaki, Japan. Stored serum samples collected in 1968–1970 were assayed for 72 women subsequently diagnosed with breast cancer and 150 control subjects in 72 case-control sets matched on age, date of blood collection, exposure, radiation dose, and city. Serum levels were determined for sex hormone binding globulin, total estradiol (E_2), bioavailable E_2 , dehydroepiandrosterone sulfate, and prolactin. Matched case-control comparisons of hormone levels were carried out by conditional logistic regression and were adjusted for menopausal status at the time of blood drawing. The odds ratio per unit log change in bioavailable E_2 was 2.2 [95% confidence interval (CI), 1.02–5.3] for all subjects, and 2.3 (95% CI, 0.55–6.8) and 2.1 (95% CI, 0.55–9.7), respectively, based only on premenopausal or postmenopausal serum. The estimated odds ratios in each quintile of bioavailable E_2 level, using the lowest quintile as referent, were 1.00, 1.89, 1.43, 3.45, and 3.37 (P for trend = 0.035). For sex hormone binding globulin, the overall odds ratio was 0.58 (95% CI, 0.14–2.26), and 1.00 (95% CI, 0.19–5.45) and 0.21 (95% CI, 0.02–1.88) based

on premenopausal and postmenopausal serum, respectively. This study offers further prospective support for the hypothesis that a high level of biologically available E_2 is a risk factor for the subsequent development of breast cancer.

Introduction

Endogenous hormones are believed to play a possibly important role in breast cancer etiology (1). However, comparisons of hormone levels between cases and controls using serum collected after diagnosis are susceptible to serious bias if the disease itself might affect hormone metabolism. A prospective study design in which serum samples from healthy women are stored and the women are followed for disease onset avoids this bias and provides direct evidence on the role of hormones in cancer risk.

RERF⁴ and its predecessor, Atomic Bomb Casualty Commission, in Hiroshima and Nagasaki, Japan have studied mortality and morbidity in a large study cohort of atomic bomb survivors and have solicited a subcohort (AHS) for biennial clinical examinations since 1958. Serum samples have been collected, frozen, and stored at Atomic Bomb Casualty Commission/RERF since the 1968–1970 examination cycle. This repository of stored samples and clinical records provides for focused studies of cancer cases and appropriately chosen controls using serum samples collected before disease onset.

We report here the results of a nested case-control study of serum hormones collected prior to breast cancer development. The prior hypothesis of primary interest was that high levels of biologically available E_2 in serum may predispose women to develop breast cancer. The serum constituents assayed were total E_2 , SHBG, bioavailable E_2 (not bound to SHBG), prolactin, progesterone, and DHEA-s. Case-control differences were evaluated for each hormonal component, alone and in combination with other risk factors including reproductive history, other medical history, and radiation dose.

Materials and Methods

Study Population. A supplement to the 1950 Japanese census identified A-bomb survivors in the two cities. From this supplement, 94,000 atomic bomb survivors who were Hiroshima or Nagasaki residents on October 1, 1950 and 26,000 city residents not exposed (not in either city on the day of the bombing) were included in the Life Span Study sample. The AHS clinical subcohort, originally numbering 8,000 male and 12,000 female subjects, was established from the larger cohort in four groups of equal size: group 1, all (or virtually all)

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⁴ The abbreviations used are: RERF, Radiation Effects Research Foundation; SHBG, sex hormone binding globulin; DHEA-s, dehydroepiandrosterone sulfate; E_2 , estradiol; AHS, Adult Health Study; OR, odds ratio; CI, confidence interval.

persons exposed at distances <2 km and who reported acute radiation injury; group 2, persons exposed <2 km who reported no radiation injury; group 3, persons exposed at 3–4 km in Nagasaki or 3–3.5 km in Hiroshima; and group 4, cohort members 10 km or more from ground zero, including the unexposed. Groups 2–4 were probability sampled and matched to group 1 by sex and age.

Participation in the biennial AHS clinical examination program has been consistently ~85% of the surviving subcohort members still resident in the two cities. Clinical examinations are given at RERF or at home if the subject is physically unable to come to RERF. The examinations involve clinical history, physical examination, blood work-up, and, if held at RERF, chest X-ray or other radiography as appropriate. Beginning with the 1968–1970 examination cycle, serum samples have been frozen and stored at -60° to -70° C, except for a 4-day period in July 1982, when the freezer malfunctioned and the temperature eventually reached 25–35°C.

Individual, organ-specific radiation dose estimates have been calculated for most (86%) exposed cohort members using the dosimetry system (DS86) introduced in 1986 (2) that replaced the T65D system introduced in 1965. Both systems were based on individual shielding histories and mathematical models for radiation yield from the bombs and its attenuation over distance and by materials and tissue. Cancer cases are identified by death certificate and through the RERF Tumor Registry (3). Diagnosis date was confirmed for all cases in the current study on the basis of pathology or clinical records. Three cases were diagnosed in 1973, five in 1974, and the remainder in 1975 or later.

Selection of Cases and Controls. All women in the AHS subcohort diagnosed with breast cancer between 1973 and 1983 and recorded as having at least 1.0 ml of stored serum from the 1970 to 1972 examination cycle were selected from the RERF tumor registry and mortality files. Initially, two control women satisfying the same sample and serum availability criteria and matched with respect to city, age (± 3 years), date (± 3 months) of blood collection, and radiation dose in Gy (nonexposed/ $<0.01/0.01$ – $0.49/0.50$ – $0.99/\geq 1.00$ /unknown dose) were selected for each case. A control woman must have been alive and cancer free at the date of case diagnosis. Cases and controls were matched on dose to remove dose as a possible confounder and (more importantly) to improve power for analyses of possible interactions between hormonal effects and radiation dose (4). Case and control selection for this study was conducted in 1985, prior to the availability of DS86 estimates. Matching was therefore based on breast tissue dose according to the T65D system, which is highly correlated with the DS86 dose.

Subsequently, based on visual inspection of the samples, 12 cases and about twice as many controls were found to have insufficient volumes of serum (*i.e.*, <1.0 ml) and were dropped from the study. Later, based on results from hormone assays, 5 cases and 10 controls were dropped because of suspected pregnancy at blood drawing. For 1 additional case and 4 additional controls, total E_2 could not be evaluated, and these subjects were dropped from analysis. Seventy-two breast cancer cases with 150 controls remained. Because losses of cases and controls left some of the original matched sets without a case or control, a modified version of the original matching criteria was applied to the remaining cases and controls without knowledge of assay results. The modified criteria involved a relaxation of the criterion for matching on radiation dose to allow exposed subjects without DS86 dose estimates to be included in matched sets of exposed subjects if other matching criteria were met.

This procedure resulted in 72 new matched sets, 52 of which had 1 case and 2 controls, and the remainder had a single case and 1, 3, 4, or 5 controls.

Laboratory Analysis. Hormonal assays were carried out by one of us (M. K.) at Nagasaki University in 1986 under blind conditions, using samples (including control samples) identified only by number. The choice of assays was governed both by the study purposes and the limited serum available for most subjects. RIA was performed using commercially available kits to determine E_2 levels (Dai-ichi Radioisotope Institute) and prolactin and progesterone (Amersham International Plc). Levels of bioavailable E_2 were calculated by multiplying total (*i.e.*, free plus albumin-bound plus SHBG-bound) E_2 values by percentage of bioavailable E_2 , which was estimated using a modified version of the charcoal method reported by Vermulen *et al.* (5).

The intra-assay coefficients of variation based on control pools were 9.8% for E_2 , 5.3% for SHBG, and 7.5% for progesterone. In a pilot study on 10 males and 10 females, repeated serum samples were collected for four periods between 1969 and 1983, and very good stability of the mean values was obtained for DHEA-s, prolactin, and E_2 . All assays were performed blind. Assay materials from several lots were mixed and applied to all subjects to avoid kit-to-kit variation.

DHEA-s levels were measured by RIA for 11-deoxy-17 ketosteroid with DHEA-s as standard. Antisera for this assay was obtained from the Third Department of Internal Medicine of the University of Tokyo. SHBG was measured by RIA kit using a specific monoclonal antibody (Farnos Diagnostica, Oulansalo, Finland).

Information from RERF Data Files. Blood pressure, height, weight, and cholesterol levels were measured at the time of blood collection and included in the clinical record of each subject. Obstetrical-gynecological histories were gathered from various RERF files, including medical records, interviews at the RERF clinics in the mid 1960s, 1978 mail survey data files (6), X-ray film records, and PAP smear records (7). Variables of particular interest as possible risk or modifying factors include number of deliveries, age at first marriage, age at first delivery, cumulative months of lactation, age at menarche, age at menopause, and smoking history.

There were seven subjects whose ages at menopause, *i.e.*, 1 year after the last menstrual period, were uncertain. These women were all categorized as postmenopausal for events (diagnosis or blood drawing) occurring after 55 years of age. One case, diagnosed at age 46, was treated as premenopausal, and one control, examined at age 44, was treated as premenopausal at blood drawing. Also, the exact timing of cancer diagnosis relative to menopause was difficult to determine for some cases. In the analysis, cases diagnosed within 1 year after menopause were treated as premenopausal cases.

Statistical Analysis. Measured levels of total E_2 , SHBG, prolactin, and bioavailable E_2 were transformed to their logarithms (base 10) because the distributions of the transformed values were more nearly symmetric than in the original scale, whereas DHEA-s and progesterone were analyzed in the original scale.

The primary analyses were comparisons of serum hormone levels between cases and individually matched controls, using the PECAN algorithm for conditional logistic analysis from the Epicure package of generalized regression programs for epidemiological data analysis (8). The results changed only slightly when analyses were conducted using a conventional logistic analysis that ignored the matching scheme but adjusted for age and the DS86 breast dose. In all regression analyses, the

Table 1 Serum hormone levels among controls, by menopausal status at blood drawing

Measurement	Units	Mean (SD)		<i>P</i> ^a for difference
		Premenopausal (n = 94)	Postmenopausal (n = 56)	
Bioavailable E ₂	log ₁₀ pg/ml	1.54 (0.37)	0.83 (0.40)	<0.0001
Total E ₂	log ₁₀ pg/ml	1.94 (0.31)	1.28 (0.25)	<0.0001
SHBG	log ₁₀ ng/ml	1.76 (0.19)	1.79 (0.25) ^b	0.42
DHEA-s	μg/ml	0.58 (0.23)	0.40 (0.23)	<0.0001
Prolactin	log ₁₀ ng/ml	1.07 (0.09)	1.01 (0.08)	0.0001

^a Based on Student's *t* test.^b n = 55.

logarithm of the OR was modeled as a linear function of the hormonal variable of interest, with separate intercepts for premenopausal and postmenopausal serum. Slope coefficients were calculated both with and without regard for menopausal status at the time of blood drawing, *e.g.*,

$$\log(\text{odds ratio}) = \alpha_1 I_1 + \alpha_2 I_2 + \beta X$$

or

$$\log(\text{odds ratio}) = \alpha_1 I_1 + \alpha_2 I_2 + (\beta_1 I_1 + \beta_2 I_2) X$$

where α_1 , α_2 , β , β_1 , and β_2 are unknown parameters, X is the variable of immediate interest, and I_1 and I_2 are indicator functions for premenopausal and postmenopausal serum, respectively ($I_1 = 1$ and $I_2 = 0$ for premenopausal serum, and $I_1 = 0$ and $I_2 = 1$ for postmenopausal serum).

In other analyses, X was replaced by a categorical variable (Y , Y_1 , or Y_2) with levels denoting placement of the observed value by quintile among observations based on assays of all serum (Y), premenopausal serum only (Y_1), or postmenopausal serum only (Y_2). *P*s presented correspond to likelihood ratio tests.

Results

Baseline Levels. Mean age of the 72 cases at examination was 48.6; that of the 150 controls was 49.0. Of the 72 cases, 14 were premenopausal and 58 postmenopausal at cancer diagnosis (mean ages at diagnosis were 50.4 and 68.7, respectively). Means, SDs, and value ranges among controls from the various hormonal assays are shown in Table 1, by menopausal status at examination. Missing values include one control for whom SHBG could not be evaluated. With that exception and the subjects excluded from the analysis for other reasons, it appears that serum hormone levels accord well with normal ranges and did not deteriorate under storage conditions, including the 4 days of freezer malfunction in 1982. Correlations between pairs of hormones are shown in Table 2, separately by menopausal status at examination. As expected, bioavailable E₂ and SHBG were inversely related in both premenopausal and postmenopausal serum.

Case-Control Comparisons: Hormone Levels. Table 3 shows the ORs estimated from conditional logistic regression (using PECAN) for bioavailable E₂, SHBG, DHEA-s, total estrogen, and prolactin. These ORs apply to a unit change in the logarithm or, in the case of DHEA-s, the untransformed level of the hormone in question. Bioavailable E₂ was significantly associated with risk (OR, 2.24 with 95% CI, 1.02–5.30); separate estimates for premenopausal and postmenopausal serum were similar, but with wider confidence limits. For SHBG, the

overall OR was 0.58 (95% CI, 0.14–2.26), and 1.00 (95% CI, 0.19–5.45) and 0.21 (95% CI, 0.02–1.88) based on premenopausal and postmenopausal serum, respectively. Total E₂ was suggestively related to risk [OR, 2.22 (95% CI, 0.85–6.17); however, there was no association after adjustment for bioavailable E₂ (adjusted OR, 1.05 (95% CI, 0.20–5.16)). Prolactin was not related to subsequent breast cancer risk. Risk increased with increasing DHEA-s, but not significantly ($P = 0.15$).

Bioavailable E₂ was divided into quintiles, and risk was estimated in each quintile relative to the first. Overall, risk increased for increasing quintile, significantly so for all serum and for premenopausal serum alone, but nonsignificantly for postmenopausal serum (Table 4).

The minimum time from blood draw to diagnosis was 2.0 years; the maximum was 13.6. However, no consistent pattern was observed in the predictive value of either bioavailable E₂ or SHBG with respect to time (data not shown). Adjustment for body mass index, age at menarche, luteinizing hormone, and progesterone level did not alter analysis results.

Case-Control Comparisons: Epidemiological Variables. Analyses with respect to epidemiological variables provided little insight into the relationships between breast cancer risk and serum hormone levels. In these data, having delivered a child (OR, 0.26; 95% CI, 0.10–0.63; $P = 0.006$) and cumulative lactation (OR, 0.49 per year; 95% CI, 0.21–0.89; $P = 0.013$) were significantly and inversely related to breast cancer risk. Information on parity was available for most subjects (67 cases and 141 controls; 66 informative data sets), but information on lactation was available for fewer than half of the subjects (32 of 72 cases and 62 of 150 controls; 20 informative data sets), and therefore, no adjustment was considered for this variable. Adjustment for parity had little or no effect on the main associations demonstrated in Tables 3 and 4. No suggestive or statistically significant associations were observed between cigarette smoking and hormone levels, nor did adjustment for smoking affect observed associations, or lack thereof, between breast cancer risk and serum hormone levels.

Discussion

The results of this prospective study support the hypothesis that estrogen availability in blood is greater prior to diagnosis in women who later develop breast cancer than in those who did not. There have been very few prospective studies of hormone levels and subsequent risk of breast cancer. Bulbrook *et al.* (9) examined 24 cases in a cohort of 5000 British women and found a higher mean serum level of bioavailable E₂ in the case group. In contrast, Wysowski *et al.* (10) and Garland *et al.* (11) did not find significant case-control differences, using 39 and 15 cases, respectively.

Toniolo *et al.* (12) conducted a nested case-control study based on 14,291 women in the prospective New York University Women's Health Study. Among 130 cases of breast cancer in postmenopausal women, they found a strong trend of increasing risk with increasing quartile of bioavailable E₂ and a strong decreasing risk with increasing quartile of SHBG-bound E₂ after adjustment for Quetelet's index. They also found positive associations of total E₂ and estrone with risk. In addition, they found a strong negative correlation of Quetelet's index with percent SHBG-bound E₂. Similarly, Key *et al.* (13) conducted a prospective study of urinary estrogens and subsequent risk of breast cancer in 1000 women. Among 31 postmenopausal cases, there was a statistically significant association of urinary E₂ concentration and of total estrogen concentration and subsequent risk. Among 38 premenopausal

Table 2 Correlations (two-tailed *P*s in parentheses) between hormone levels for cases and controls, based on assays of premenopausal serum (data above 1.00 value) and postmenopausal serum (data below 1.00 value)

	Bioavailable E ₂	SHBG	Total E ₂	DHEA-s	Prolactin
Bioavailable E ₂	1.00	−0.27 (<i>P</i> = 0.001)	0.81 (<i>P</i> < 0.001)	0.22 (<i>P</i> = 0.008)	0.09 (<i>P</i> = 0.29)
SHBG	−0.59 (<i>P</i> < 0.001)	1.00	0.20 (<i>P</i> = 0.017)	−0.26 (<i>P</i> = 0.002)	0.14 (<i>P</i> = 0.09)
Total E ₂	0.74 (<i>P</i> < 0.001)	−0.14 (<i>P</i> = 0.23)	1.00	0.17 (<i>P</i> = 0.046)	0.15 (<i>P</i> = 0.08)
DHEA-s	0.22 (<i>P</i> = 0.043)	−0.20 (<i>P</i> = 0.07)	0.21 (<i>P</i> = 0.06)	1.00	−0.06 (<i>P</i> = 0.47)
Prolactin	−0.03 (<i>P</i> > 0.05)	−0.07 (<i>P</i> > 0.5)	−0.04 (<i>P</i> > 0.5)	−0.06 (<i>P</i> > 0.5)	1.00

Table 3 Estimated ORs (95% CIs) per unit increment in (log₁₀^a) hormone level

Serum (cases/controls)	Bioavailable E ₂	SHBG	Total E ₂	DHEA-s ^a	Prolactin
All ^b	2.24	0.58	2.22	2.61	1.76
(72/150)	(1.02–5.30)	(0.14–2.26)	(0.85–6.17)	(0.70–9.94)	(0.02–43.9)
Premenopause	2.30	1.00	3.57	2.40	1.01
(46/94)	(0.85–6.78)	(0.19–5.45)	(1.07–13.4)	(0.47–12.3)	(0.02–47.4)
Postmenopause	2.13	0.21	0.77	3.06	6.45
(26/56)	(0.55–9.69)	(0.02–1.88)	(0.11–5.06)	(0.34–30.7)	(0.01–43.9)

^a DHEA-s in original scale.

^b Adjusted for menopausal status at blood drawing.

Table 4 Estimated OR (95% CI) in each quintile of bioavailable E₂

Serum	1st (reference)	2nd	3rd	4th	5th	<i>P</i> (trend)
All ^a	1.00	1.89	1.43	3.45	3.37	0.035
		(0.69–5.73)	(0.41–5.30)	(1.02–13.4)	(1.00–13.2)	
Premenopause	1.00	1.05	1.87	2.62	3.05	0.009
		(0.30–3.90)	(0.54–7.17)	(0.79–9.91)	(0.91–11.7)	
Postmenopause	1.00	2.12	5.25	7.35	2.52	0.17
		(0.40–16.9)	(0.72–64.5)	(1.20–78.7)	(0.19–40.2)	

^a Adjusted for menopausal status at blood drawing.

cases, there were no significant associations. Two more prospective studies reported an association of biologically available E₂ and risk and strong associations of androgens and risk (14, 15). The studies were methodologically strong but had small numbers of cases (71 and 25, respectively). In Dorgan *et al.* (14), the association with androgens was restricted to cases occurring within 2 years of blood draw.

Three recent studies have reported associations of elevated serum E₂ with risk of postmenopausal breast cancer (16–18). Cauley *et al.* (16) studied 97 women with breast cancer from a cohort of 9704 women of ages 65+ in the United States. They reported a relative risk of 3.6 for women in the highest quartile of bioavailable E₂ compared with the lowest quartile. They also found a strong association of free testosterone and risk; however, this did not attain statistical significance after adjustment for bioavailable E₂. Hankinson *et al.* (17) used the Nurses' Health Study to examine plasma hormone levels and risk of breast cancer in the United States. Among 156 women diagnosed over the study period, 1989–1994, total E₂, estrone, estrone sulfate, and DHEA-s were each significantly associated with risk. Bioavailable E₂ and testosterone were also marginally significant. In multivariate analyses, adjustment for total E₂ substantially reduced risk associated with testosterone, and it became nonsignificant. Thomas *et al.* (18) reported a strong association of total circulating E₂ concentration and breast cancer risk in a nested case-control study of 61 cases and 179 control chosen from within a prospective follow-up of 6127

women from the island of Guernsey in the United Kingdom (OR, 5.0 for highest tertile compared with lowest). E₂ was strongly associated even after adjustment for testosterone and SHBG; however, after adjustment for E₂, testosterone and SHBG were not significantly associated with risk. In a review of epidemiological studies published from 1966 to 1996, Thomas *et al.* (19) reported that the results from prospective studies supported an association of high total E₂ (serum or urinary, depending upon the study) and risk of breast cancer in postmenopausal women. There was no significant heterogeneity among these studies.

In the present study, we distinguished between cases who had serum drawn before and after menopause because of the impact of menopause on hormone levels. In this prospective study, cases with postmenopausal serum were all diagnosed after menopause, whereas among the women with premenopausal serum, there were cases diagnosed both before and after menopause. There were too few cases diagnosed before menopause to be separately informative about the association of hormones and risk.

Unfortunately, no record was kept of stage of menstrual cycle at time of clinical examination, nor was time of day recorded for blood drawing. The problem here is not a possible bias attributable (for example) to an association between subsequent development of breast cancer and stage of the menstrual cycle at time of blood drawing, but one of reduced ability

to do refined statistical analyses with respect to hormone levels in premenopausal serum.

We were concerned about the reliability of the measurement of bioavailable E_2 in sera that had been stored for as long as 15+ years. We were encouraged by the facts that: (a) age-specific levels of bioavailable E_2 among control subjects were comparable with those reported by other researchers; and (b) bioavailable E_2 levels showed a strong positive correlation with total E_2 and strong negative correlation with SHBG (Table 2). There is high variability among women in total E_2 level (20) and lower levels in Japanese than American women (21). Given this variability, our values are in the same range as studies published previously.

A case-control interview study (4, 22), based on 196 cases and 566 controls from the full cohort of the RERF Life Span Study, reported a strong, positive association of risk with age at first full-term pregnancy. Inverse associations were observed for number of births and total period of breastfeeding, even after adjustment for age at first birth. The present study was conducted separately, with subjects drawn from those members of the Life Span Study clinical subsample for whom stored serum was available. However, the findings with respect to epidemiological factors were comparable with respect to those in the interview study given the smaller numbers of cases and controls. The interview study also investigated interactions between radiation dose and reproductive factors and found a generally multiplicative relationship; for example, early age at first full-term pregnancy was found to be protective against both baseline and radiation-related breast cancer to about the same degree. The relationships between risk and serum hormone levels were not sufficiently clear in these data to yield clear results with respect to interaction with radiation dose.

In summary, our study showed a positive association of bioavailable E_2 with risk of breast cancer in Japan, despite the fact that Japanese women have lower bioavailable E_2 and higher SHBG than Caucasian women (21).

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